

Measurement of DNA Adducts for the Assessment of DNA Damage Repair in Cancer

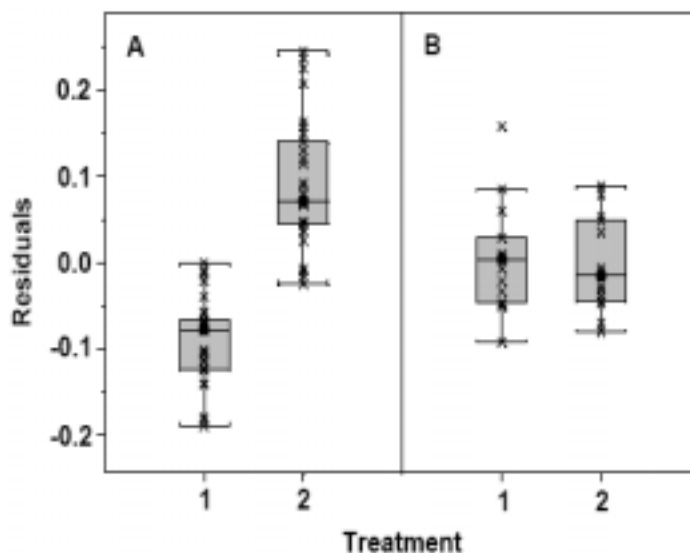
NIST scientists have developed a method to detect defective DNA damage repair mechanisms in cells from cancer patients. Defective DNA repair is a hallmark of cancer and may be a good indicator for the presence of gene mutations such as BRCA1 and BRCA2 in breast cancer. This method is based upon the ability of blood cells from cancer patients to withstand irradiation by repairing characteristic DNA lesions, and is expected to enable innovation of new cancer treatments and diagnostics.

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Breast cancer is the second leading cause of cancer deaths among women. Inherited mutations that affect a single allele of the breast cancer 1 and 2 genes (BRCA1 and BRCA2) predispose women to high risk of breast and ovarian cancers, although the magnitude of this risk is controversial. Hereditary breast cancers accounts for approximately 5-10% of all breast cancers among women, whereas other breast cancers are considered to be sporadic. On the other hand, 30-60% of familial breast cancers result from inherited mutations on BRCA1 and BRCA2. BRCA1 plays an important role in maintaining the genome integrity, at least in part, through its role in the repair of DNA damage. Thus, the BRCA1 product, BRCA1 is involved at multiple steps in the cellular response to DNA. Further evidence shows that BRCA1 plays a role in the repair of other types of oxidative DNA damage as BRCA1-deficient cells exhibit chromosomal abnormalities and are hypersensitive to oxidative damage caused by DNA-damaging agents such as ionizing radiation and hydrogen peroxide. In this work, we investigated the cellular repair of several oxidatively induced DNA lesions in lymphoblasts of women with BRCA1 mutations in comparison to those of women with no detectable BRCA1 deficiency.

Mutations in breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* predispose women to high risk of these cancers. NIST's DNA damage repair test may enable better understanding of the fundamental issue in breast cancer.

Here, we show that lymphoblasts of women with *BRCA1* mutations, who had been diagnosed with breast cancer, are deficient in the repair of some typical products of oxidative DNA damage, namely 8-hydroxy-2'-deoxyguanosine and 8,5'-cyclopurine-2'-deoxynucleosides. Cultured lymphoblasts from 10 individuals with *BRCA1* mutations and those from 5 control individuals were exposed to 5 Gy of ionizing radiation to induce oxidative DNA damage and then allowed to repair this damage. DNA samples isolated from these cells were analyzed by using measurement methods developed in our laboratory. We used liquid chromatography/mass spectrometry and gas chromatography/mass spectrometry to measure 8-hydroxy-2'-deoxyguanosine, (5'S)-8,5'-cyclo-2'-deoxyadenosine, (5'R)-8,5'-cyclo-2'-deoxyguanosine and (5'S)-8,5'-cyclo-2'-deoxyguanosine. After irradiation and a subsequent period of repair, no significant accumulation of these lesions was observed in the DNA from control cells. In contrast, cells with *BRCA1* mutations accumulated statistically significant levels of these lesions in their DNA, providing evidence of a deficiency in DNA repair. In addition, a commonly used breast tumor cell line exhibited the same effect when compared to a relevant control cell line.



Levels of 8-hydroxy-2'-deoxyguanosine in DNA of lymphoblasts from women with BRCA1 mutations and from control women. A: Cells with BRCA1 mutations; B: Control cells, 1: Non-irradiated, 2: Irradiated at 5 Gy.

The data suggest that *BRCA1* gene product BRCA1 plays a role in cellular repair of oxidatively induced DNA lesions. The failure of cells with *BRCA1* mutations to repair 8,5'-cyclopurine-2'-deoxynucleosides indicates the involvement of BRCA1 in nucleotide excision repair of oxidative DNA damage. This work suggests that accumulation of these lesions may lead to a high rate of mutations and to deleterious changes in gene expression, increasing breast cancer risk and contributing to breast carcinogenesis. The measurement of oxidative DNA damage and its repair in human tissues will contribute to the understanding the role of BRCA1 in risk assessment for breast cancer and may lead to development of therapeutics for prevention and treatment. We plan to further investigate the effect of BRCA1 on the repair of oxidative damage to DNA in human tissues and measure other major lesions of DNA damage.

Publications:

Rodriguez, H., Jaruga, P., Nyaga, S. G., Evans, M. K. and Dizdaroglu, M., **“Lymphoblasts of women with *BRCA1* mutations are deficient in cellular repair of 8,5'-cyclopurine-2'-deoxynucleosides and 8-hydroxy-2'-deoxyguanosine,”** *Biochemistry* (in press).